A Synopsis on

The Proposed Research Work Leading to the Degree of

Doctor of Philosophy in

Botany

Study of Epidemiology and Management of Some Fungal Diseases of *Macrotyloma uniflorum* (Lam.) Verdc.

Submitted to

Swami Ramanand Teerth Marathwada University, Nanded

Submitted by

Mr. Sadashiv Nivratirao Shinde

Under the Guidance of

Dr.Venkat S. Maske

Department of Botany

B.S. College, Basmatnagar, Dist. Parbhani

1. INTRODUCTION:

Horse gram (*Macrotyloma uniflorum*, previously *Dolichosbiflorus*) an underexploited legume of tropics and subtropics grown mostly under dry-land agriculture. Horse gram is popularly called as "Madras Bean" due to the principal cultivation and rural production of this legume before the separation of these states from Madras Presidency state, India. It is an important source of protein and identified as one of the potential food sources for the future by the US National Academy of Sciences (1979). Horse gram is however, consumed as sprouts in many parts of India. Due to its good nutritional profile, new food products are being formulated to serve as a supplementary food for the nutritionally vulnerable communities.

The use of raw horse gram and its flour is well known in recent days, maximum utilization is lacking due to tannin, trypsin inhibitor, phytic acid and bowman-birk inhibitors as antinutritional factors which interfere with the bioavailability of nutrients present in horse gram (Kumar et al., 2002). However, notable progression has been achieved through dehulling, germination, fermentation, dehydration, soaking and partial hydrolysis of proteolytic enzyme to reduce the antinutrient factors.

1.1 Global cultivation, production and Condition:

Horse gram is native to tropical southern Asia and has been found in archaeological sites in India, starting from 2500 BC. It was probably first domesticated in India, and is now grown as a legume from India to Myanmar. It is also grown for fodder and as a green manure crop in tropical countries in southeastern Asia, and in northern Australia.

Horse gram is mainly cultivated in the states of Karnataka, Andhra Pradesh, Orissa, Tamil Nadu, M.P., Chhattisgarh, Bihar, West Bengal, Jharkhand, and in foot hills of Uttaranchal and Himachal Pradesh, in India. It is also cultivated in other countries mainly like Sri Lanka, Malaysia, West Indies etc. During Twelfth Plan (2012-2015) in India, the total area under Horse gram and its production during this plan was 2.32 lakh hectares and 1.05 lakh tonnes respectively.In terms of area and production, Karnataka is on the first position on all India basis contributing 26.72% and 25.71% respectively followed by Odisha (19.46%& 15.48%)and Chhatisgarh (19.29% & 13.29%).The highest

yield was recorded in the state of Bihar (959 kg/ha) followed by West Bengal (796 kg/ha) and Jharkhand (603 kg/ha) (DES, 2015-16).

1.2 Causal agent:

Several diseases caused by some fungal agents are known to attack *Macrotyloma uniflorum* plants known to be susceptible to the infection of wide range all over the world.

The most common and destructive fungal agents of Horse gram are *Colletotrichum spp*. (Sharma, 1976), *Cercospora spp.* And *Rhizoctonia bataticola* are the most isolated fungi from roots and leaves of Horsegram, (Saxena, 2017).

2. REVIEW OF LITERATURE:

To retrospective the works already done in the field of some fungal diseases and biological control managements by different workers; for enabling us to understand the research problem properly; review of literature is cited here with, would help us to frame a sound background for present study..

2.1 *Colletotrichum:*

Colletotrichum higginsianum is an important hemibiotrophic fungal pathogen that causes Anthracnose disease on various plants (Narusaka et al., 2004; O' Connell et al., 2004).The pathogen involving *C.higginsianum* and *Arabidopsis thaliana* is an attractive model to examine fungal plant interactions strategy by the pathogen (Yan et al., 2018). On the host plant, a set of specializedinfection structure is generated by the fungus, consisting of germ tubes, appressoria, biotrophic hyphae, and necrotrophic hyphae (O'Connell et al., 2012).

2.2 *Rhizoctonia:*

The genus *Rhizoctonia* was originally based on sterile mycelium attacking alfalfa roots, the name means ' root killer (Baker, 1970*). Rhizoctonia solani* Kuhn causes more different types of diseases to a wider variety of plants, over a larger part of the world and under more diverse environmental conditions than any other fungi (Baker, loc. cit.)

2.3 *Cercospora spp:*

Once the disease has been developed, the pathogen can survive in the plant debris as dormant mycelium. The pathogen produces conidia in lesions of plant debris (Payne and Waldron, 1983). These conidia play a role of primary inoculums in disease incidence. Rain splashes also play a major role in dispersal of conidia (William, 1987*). Cercospora* leaf spot is considered as an important pathogen not only due to its widespread range but also due to the susceptibility of leaves may become unshaped and wrinkled

Biological Formulations:

Biological control, in the broadest sense could be defined as the use of living agents to control pests, plant pathogens and weeds. To achieve this, appropriate formulations are required which can amplify the intrinsic properties of biocontrol agents (BCA). Formulation is also necessary in order to present the BCA in a usable form and also to make the end product efficacious, stable and easy to handle. (Majumder, 2000).

Roco and Perez, (2001), reported that Biocontrol ability of *Trichoderma* species is known since long time as they produce certain secondary metabolites against pathogen and gives resistance to abiotic and biotic stress.(Meena et al. 2004); also reported that application with *T.viride* after 45 DAS (days after sowing) reduced the incidence of blight disease of mustard plant as efficiently as chemical fertilizer mancozeb. Use of *Trichoderma harzianum* (UPM40) not only improvise soil but also significantly control the growth of damping off pathogens in *Brassica rapa* L

Abdul-Aziz et.al (2010), studied on antifungal activity of ethanol-water extracts of four medicinal plants, cinnamon (*Cinnamomum verum* Presl.), anise (*Pimpinella anisum* L.), black seed (*Nigella sativa* L.) and clove (*Syzygium aromaticum* L. Merr. & Perry.) and investigated against root-rot fungus *Rhizoctonia solani*. *In vitro* antifungal activity test shown a high growth inhibition at concentration (4%) of each plant extract..

Even though, nowadays researchers are more interested in biological control agents and their antifungal metabolites due to the development of resistance in the pathogen. Biological control is an alternative approach to the chemical fungicides and it may be a safe, effective and ecofriendly method for plant disease management.

3. GAP IN THE EXISTING RESEARCH:

Natural products seems to be a viable solution to the environmental problems caused by synthetic fungicide and many researchers are trying to identify the effective natural products for the control of diseases but very less studies have been reported on the use of phytoextracts from some common weed plants against fungal disease management in *Macrotyloma uniflorum.*

The present study is designed for the biological control of *Macrotyloma uniflorum*by using weed plant species *In vitro andin vivo* as raw material for plant derived fungicides, can manage the disease, and at the same time might create economic uses for these unwanted species

4. SIGNIFICANCE OF THE STUDY:

Nowadays many more fungicidal agents available in the market are very toxic and have undesirable effects on other organisms present in the environment. Some synthetic fungicides are non- biodegradable, and hence can accumulate in the soil, plant and water and consequently affect the humans and other living animals through food chain. So that, this study will help us for the use of some organic green manure, foliar spray and ecofriendly measures for the management of diseases.

5. OBJECTIVES:

1. To isolate and characteerice of fungal pathogen from infected plant of *Macrotyloma uniflorum*.
2. To identify secondary metabolites produced by isolates.
3. To evaluate of different biocontrol agents like*Trichoderma*from soil and plant material.
4. To study antifungal activity of selected phytoextract on fungal pathogens.
5. To study effect of fungicides on the yield of the *Macrotyloma uniflorum*plant.

6. HYPOTHESIS/ASSUMPTIONS:

- Soil rhizosphere is rich in diverse types of microorganisms. Some of them are harmful (pathogenic), i.e., responsible for infecting plant parts thereby reducing the

netyield. Also few of them helps enhancing crop yield by secreting plant growth promoting substances also known as Plant Growth Promoting Rhizobacteria(PGPR)**.**

- Find out cost effective alternative to the fungicide and fertilizers.
- Identification of rhizosphere microorganisms for their potential to use against the causative agent of fungal disease in *Lepidium sativum*.
- Parameter optimization of selected isolates to enhance its potency.
- Morphological and biochemical characterization of the isolates.
- Molecular identification using isolates by DNAsequencing.
- Analysis of secondary metabolites from different plant extracts to identify the fungicides from plant material using method GCMS.
- *In vivo* studies on *Macrotyloma uniflorum*L.

7 RESEARCH METHODOLOGY:

Material

Lab Study

- The research studies will be carried out in the Department of Botany, J.E.S. College, Jalna.
- For DNA sequencing (DNA barcoding lab, Department of Zoology Dr. Babasaheb Ambedkar Marathwada University, Aurangabad or any standard/national institute).

Collection of Materials

1. *Macrotyloma uniflorum* seeds will be collected from local fields.
2. Infected plant material of *Macrotyloma uniflorum* plant will be collected from fields. The infected plant material will be brought to the laboratory in sterile plastic bags.

Instruments required

1. Thermal cycler (Polymerase chain reaction)
2. DNA sequencer (DNA barcoding lab Dr. BAMU,Abad)
3. Spectrophotometer
4. Coolingultra-centrifuge
5. Agarose gel electrophoresisapparatus

6. Poly acryl amide gel electrophoresis apparatus
7. HPLC (from any national or standard research center)
8. Refrigerator
9. Laminar air flow
10. Compoundmicroscope
11. Incubator
12. Soxhlet extractor
13. Flame Photometer
14. Micro Kjeldahl Apparatus

Methodology

1. **Isolation and identification of *some fungi* from infected part of** *Macrotyloma uniflorum* **plant and its rhizosphere soil**.
2. **Isolation of biocontrol agents from soil rhizosphere by agar plating method**.
    2.1 Pure culture of microorganisms (Harley, J.P. and Prescott, L.M., 2002)
    2.2 Laboratory screening of isolates for antagonistic activities against *fungal pathogen.*
    2.3 Mix culture technique
3. **Identification and Characterization of isolates**
    3.1 Morphological identification (Harley and Prescott, 2002)
    3.2 Biochemical characterization of isolates (Harley and Prescott, 2002)
    3.3 Molecular characterization
4. **Analysis of secondary metabolites production by isolates.**
5. **Bioformulation and *in vivo* studies**
    5.1 Development of bio-formulation of antagonistic and plant growth promoting isolates.
    5.2 Effect of biocontrol agents on fungal diseases on *Macrotyloma uniflorum*.
    5.3 *In vivo* management of fungal disease.
6. **Induction of defense related compounds in** *Macrotyloma uniflorum* **plant**
7. **Statistical analysis**

    All data will be statistically analyzed using the standard method 'Analysis of Variance' (Mungikar. 1997) Software.

8. TENTATIVE CHAPTER'S SCHEME:

1. Introduction
2. Review of literature
3. Materials and Methods
4. Results and discussion
5. Conclusion
6. References

9. OUTLINE OF RESEARCH WORK:

Period of Study	Research Plan
First Year	
00 – 03 Months	In the first phase, reference work will be carried out in order to get familiar with work done in this research topic. Literature will be collected from research papers, articles, reviews, from journals, conferences, books, proceedings, Internet (Google scholar, science direct, American Chemical Society Journals, Springer journals, Wikipedia, web of science) resources.
04 - 08Months	Isolation and establishment of fungal disease casual organisms. Characterization, identification and maintenance of pure culture. Also to check pathogenicity of the isolated pathogens.
	***In vitro* and *in vivo* studies of fungal pathogens.**
09 - 12Months	To study biochemical profile of the plant in three stages: • Before infection • At the time of infection • After infection
13 -17 Months	**Second Year**

	Effect of biocontrol agents on fungal diseases of *Macrotyloma uniflorum.* *In vivo* management of fungal disease.
18 - 24 Months	**Analysis of secondary metabolites of isolates**. **Bioformulation and *in vivo* studies** • Development of bio-formulation of antagonistic and plant growth promoting isolates.
Third Year	
18 - 22Months	**To find out biocontrol agents against fungal pathogens.** Induction of defense related compounds in *Lepidium sativum the* plant. **Statistical analysis.** All data will be statistically analyzed using the standard method 'Analysis of Variance' (Mungikar. 1997) SOS and SOFA software.
23-28Months	• This is the last phase which constitutes report writing. Experimental data and output of the experimentation will be documented and documentation will be incorporated in proper place. • Work cited and literature review surveyed from various sources will be mentioned. Details of the work will be compiled in a systematic scientific format so as to give it a shape of a thesis. • Report writing and submission of thesis.

10. REFERENCES:

Abdul Aziz, Al – Askar, (2010). Efficacy of Some Plant Extract against *Rhizoctonia Solani* on Pea*, Journal of Plant Protection Research*. Vol. 52, No.– 3.

Baker R, Martinson CA, (1970). Epidemiology of diseases caused by *Rhizoctonia solani*. In: Parameter JR, ed. *Rhizoctonia solani*: *Biology and Pathology*. Berkeley, CA, USA: University of California Press, 172–88.

Kundu, P.K., Nandi, B.(1993), Control of *Rhizoctonia* disease of cauliflower by competitive inhibition of the pathogen using organic amendments in soil. Plant Soil **83,** 357–362

Lok Bahadur Pun, Kamal Chhetri, Anju Pandey and Rajan Paudel, (2020). In Vitro Evaluation of Botanical Extracts, Chemical Fungicides and *Trichoderma harzianum* against *Alternaria brassicicola* Causing Leaf Spot of Cabbage. *Nepalese Horticulture* 14: 68-76.

Meena, P.D., Meena, R.L., Chattopadhyay, C. and Kumar, Arvind. (2004). Identification of Critical Stage for Disease development and Biocontrol of *Alternaria* Blight of Indian Mustard (*Brassica juncea*) J. Phytopathology,152: 204-209

Majumdar V.L. and Yadav R.K. (2005). Efficacy of Plant Extracts, Biological Agents and Fungicides against *Lasiodiplodia Theobromae* Causing Die Back Of Guava (*Psidium guajaya* L.). *Journal of Mycology and Plant Pathology*, 35: 352-353

Roco A, Perez LM.(**2001***).In vitro* biocontrol activity of *Trichoderma harzianum* on *Alternaria alternata* in the presence of growth regulators. EJB Electronic *Journal of Biotechnology* ISSN: 0717-3458 Vol.4 No.2.

Research Student

Research Guide

Mr. Sadashiv Nivratirao Shinde

Dr. Venkat S. Maske

Date: 14/07/2021